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A STUDY OF THE REGULATION OF THE RATE OF URINARY AMMONIA EXCRETION IN THE RAT

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There is still disagreement about the mechanism which controls the rate of ammonia excretion by the kidney. According to the classical view (Peters, 1935; Gamble, 1942) urinary ammonia formation is increased during a systemic acidosis and conserves the organism's store of fixed base. The effective stimulus causing this increase in ammonia production is generally thought to be some change in acid-base composition of the *plasma*. Evidence for an alternative hypothesis was presented originally by Briggs (1934). From the results of experiments which involved the administration of sodium sulphate and of potassium chloride he concluded that the acidity of the *urine* determined the rate of ammonia formation. More recently, Wolf (1947) has found ammonia formation to be more closely related to urinary pH than to any other physiological variable studied.

Under most conditions a change in the composition of the plasma towards a state of acidosis or alkalosis is immediately reflected by a corresponding change in the urine. Any procedure which produces an independent or discordant variation in the plasma acid-base balance and the urinary pH should help to decide between the above hypotheses. Pitts & Alexander (1945) have demonstrated that the renal tubular cells must actively transfer hydrogen ions into the urine in order to account for the observed maximal titratable acidities. Moreover, they concluded that the only source of hydrogen ions large enough for this operation is the carbonic acid of the cells and tissue fluids. This conception receives support from the observations that (1) carbonic anhydrase occurs in the renal cortex (Davenport & Wilhelmi, 1941), (2) sulphonamide inhibitors of carbonic anhydrase cause a decrease in urine acidity (Höber, 1942; Pitts & Alexander, 1944). As would be expected, this induced decrease in urine acidity results in the simultaneous development of a systemic acidosis (South-

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worth, 1937). This phenomenon has been used to obtain additional evidence on the problem of the regulation of ammonia formation.

The present communication reports the effect of variations in urinary pH induced by a carbonic anhydrase inhibitor on the ammonia production of rats. For comparison, the urinary pH and ammonia production were determined during an acute systemic alkalosis produced by administration of sodium bicarbonate or sodium carbonate. The compound *p*-sulphonamidobenzoic acid ($NH_2SO_2-C_6H_4-COOH$) was employed as the inhibitor because of the strength of its antagonism to carbonic anhydrase in the presence of tissue homogenates (Krebs, 1948).

METHODS

General procedure. Eight adult female rats, ranging from 175 to 306 g. in weight, were used in the reported experiments. Without special preparation an animal was removed from the colony on the morning of an experiment. Under light ether anaesthesia a 4F ureteric catheter was introduced into the bladder and the rat was placed in a tubular holder for the duration of the experiment. A few minutes after recovery from the anaesthetic, the animal was given 5–8 ml. of fluid (according to body weight) by stomach tube. This consisted of approximately 0·1–0·2 g. of *p*-sulphonamidobenzoic acid suspended in water in the five experiments in which carbonic anhydrase was inhibited. The fluid administered to the three rats in which a systemic alkalosis was induced was 0·154 M-$NaHCO_3$ in the first, 0·072 M-Na_2CO_3 in the second, and 0·20 M-Na_2CO_3 in the third. The exact doses are given in Tables 1 and 2. A 150 W. lamp was placed about 6 in. above the holder to prevent chilling, and the animal's eyes were shielded from the light.

Technique of catheterization. Approximately 0·30 ml. of water-soluble lubricant (KY jelly) was introduced into the urethra and bladder, using a tuberculin syringe with a blunt needle. This was easily accomplished by holding the lips of the urethral papilla snugly around the needle tip with the thumb and forefinger of the left hand while operating the plunger with the right. A small slit was then made in the urethral papilla and excess lubricant wiped away with cotton-wool. The opening of the urethra into the papillary sinus could then be directly visualized. A nickel-chrome wire fitted with a blunt glass tip made from capillary tubing was gently inserted into the urethra and advanced until the dome of the bladder was reached. The 4F catheter was threaded over the wire, and with the latter serving as a guide the catheter was advanced into the bladder. Nupercaine ointment was applied to the urethral opening and the catheter sutured in place with cotton thread. Withdrawal of the wire caused the glass tip to fall free into the bladder. After replacing the rat in the holder, the bladder was washed out with saline until the lubricant could no longer be detected in the washings.

Urine collections. The lower end of the catheter led into a small tube surrounded by ice. As soon as the diuresis started, usually in about 60–90 min., the bladder was washed out with three to five successive volumes of 0·15–0·20 ml. of 0·9 % (w/v) NaCl and the first collection period started. At the end of each collection period of 30–60 min., the bladder was similarly washed out. From three to six collections were obtained from each rat.

Urine pH *determinations* were carried out by means of a Stadie glass electrode system and a Cambridge pH meter. Carbon dioxide loss was minimized by keeping the samples undisturbed at 0° C. and by performing the determination immediately after each collection period. The temperature at which the pH determinations were carried out varied from 17·5 to 20·5° C.

Ammonia estimations. All bladder washings, together with the total urine sample and several washouts from the glass electrode system, were placed in a 50 ml. volumetric flask with 2 ml. of 1·4 N-HCl, diluted to 50 ml. and stored at approximately 4° C. until the ammonia estimations were performed. These were carried out with 1 or 2 ml. aliquots in standard Conway units, using the phenate-hypochlorite method of Russell (1944) and a Spekker photoelectric absorptiometer.

Expression of results. At the end of each experiment the rat was killed by a blow on the head. Both kidneys were then removed, decapsulated and weighed. Before these experiments began, dissection of two kidneys and separate weighings of the cortex and medulla showed that 76% by weight of the entire kidney was cortex. The weight of the cortex in each experiment was estimated by multiplying the weight of both kidneys by a factor of 0·76. Ammonia nitrogen has been expressed as μg. formed per hour per gram of moist cortex.

RESULTS

The experiments with an inhibitor of carbonic anhydrase

There were five experiments (see Table 1) in which the rats were given an aqueous suspension of *p*-sulphonamidobenzoic acid into the stomach by means of a rubber catheter. The dose ranged from approximately 40 to 90 mg./100 g. of rat. Since this material was in suspension and a variable amount remained within the catheter, the dosages listed are only approximate. It was the rule in these experiments for the urine samples to show the highest pH during the first periods of diuresis, with a progressive lowering of pH thereafter. In general, the larger doses produced a pH elevation of greater degree and duration than did the smaller ones. Fig. 1 illustrates how the ammonia production, which is represented by the solid dots, decreased as the pH was elevated by the inhibitor of carbonic anhydrase.

TABLE 1. Details of the five experiments in which the carbonic anhydrase inhibitor *p*-sulphonamidobenzoic acid was administered

Exp.	Weight of rat (g.)	Volume of fluid given in ml./100 g. of rat	Range of urine flow in ml./hr./100 g. of rat	Approx. dose of inhibitor in mg./100 g. of rat	Range of urine pH
1	222	2·7	0·23 0·41	90	7·62 7·95
2	238	2·3	0·18 0·35	40	6·69 7·76
3	210	2·7	0·16 0·23	70	6·84 8·02
4	233	2·4	0·20 0·49	40	6·28 6·66
5	244	3·1	0·21 0·37	65	7·18 7·97

Although plasma analyses were not performed, it is reasonable to assume that the animals developed some degree of systemic acidosis during those experiments in which a very alkaline urine was excreted.

The experiments in which an alkali was administered

In order to obtain a series of urine samples with a spread in pH values similar to that observed in the above experiments, alkalinizing solutions of varying concentration were given by stomach tube. The solution contained sodium bicarbonate in Exp. 6, and sodium carbonate in Exps. 7 and 8, with a

variation in total dose of 0·40–1·04 m.equiv. of alkali/100 g. of rat. This was administered in a fluid volume comparable to that used in the experiments with *p*-sulphonamidobenzoic acid.

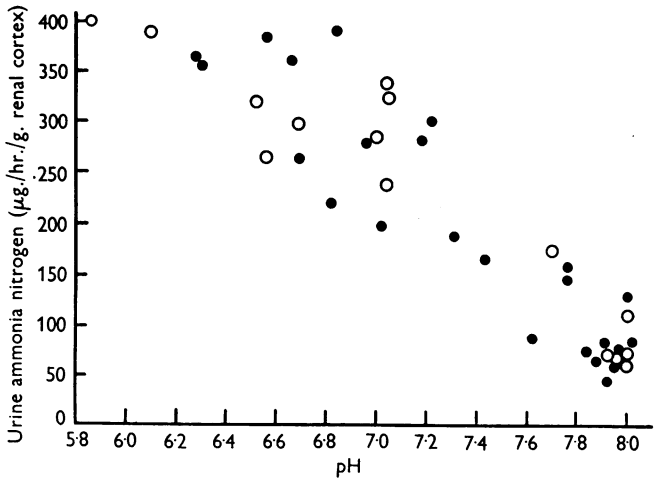

Fig. 1. Showing the excretion of ammonia nitrogen in μg. per hr. per g. of renal cortex as a function of urinary pH. The solid dots (●) represent collection periods after administration of *p*-sulphonamidobenzoic acid. The open circles (○) represent collection periods after administration of an alkali.

It will be noted (Table 2) that there was no overlapping of urinary pH values in the three experiments, and that the animal which received sodium bicarbonate (Exp. 6) continued to excrete an acid urine during three collection

Table 2. Details of the three experiments in which sodium bicarbonate (Exp. 6) or sodium carbonate (Exps. 7 and 8) was administered

Exp.	Weight of rat (g.)	Volume of fluid given in ml./100 g. of rat	Range of urine flow in ml./hr./100 g. of rat	Dose of alkali in m.equiv./100 g. of rat	Range of pH
6	241	2·7	0·12 0·24	0·42	5·86 6·52
7	306	2·6	0·29 0·62	1·04	7·70 8·00
8	175	2·9	0·30 1·10	0·40	6·56 7·05

periods. Undoubtedly the rat of Exp. 7 was in a state of systemic alkalosis with urine pH values of 7·70–8·00. The open circles in Fig. 1 give the variations in ammonia excretion as a function of urinary pH. The similarity in the distribution of these points to those obtained in the experiments with an inhibitor of carbonic anhydrase is apparent.

DISCUSSION

The data presented are in conformity with the conclusion of Wolf that the rate of ammonia excretion is primarily correlated with urinary pH. This correlation might *a priori* be due to some change *within* the cell determined by the level of activity of the mechanism for transferring hydrogen ions into the lumen. On the other hand, it may be due to the circumstance suggested by Briggs (1934) that the acidity of the urine in the tubular lumen, i.e. *outside* the cell, provides the effective stimulus to ammonia formation. Evidence which favours the latter view is provided by an experiment of Pitts & Alexander (1944). These workers measured urinary ammonia excretion in an acidotic dog before and during the intravenous infusion of a phosphate buffer solution. As the concentration of phosphate buffer in the glomerular filtrate increased, the pH of the urine rose, and so did the output of titratable acid (expressed in millimoles per minute), the latter being an index of the activity of the mechanism for transferring hydrogen ions into the urine. There was a fall in the rate of ammonia production indicating the primary importance of the *intensity* factor of urinary acidity.

It should be pointed out that the concept that ammonia formation helps to prevent the loss of fixed base during states of acidosis is not invalidated because the rate of ammonia production is determined by the pH of the urine. Anions can only be excreted in combination with hydrogen ions, fixed base and ammonia. The process of transferring hydrogen ions has quantitative limitations (Pitts & Alexander, 1944), and any excess of anions beyond this limit which require excretion must be accompanied by fixed base unless ammonia is substituted. A state of acidosis likely to be encountered outside the laboratory calls for the excretion of more anions but lowers the pH of the urine at the same time and thus stimulates the formation of ammonia to be excreted with them.

Følling's (1929) detailed study of an acidosis induced by ammonium chloride shows that urinary acidity is not the *sole* determinant of the rate of ammonia formation. On successive days following the ingestion of this acidifying salt, the ammonia excretion showed a continued rise without any corresponding increase or even with a decrease in the titratable acidity. This is true even if one takes into account the theoretical maximal acidity within the tubules before a possible partial neutralization by ammonia. Titratable acidity can be considered an index of urinary pH in Følling's experiment because his data show no appreciable change in the principal urinary buffer, i.e. inorganic phosphate.

With the administration of an inhibitor of carbonic anhydrase to an experimental animal a condition is induced which is analogous to the clinical syndrome described by Lightwood (1935) and by Albright & Reifenstein (1948). These patients show an impaired ability to elaborate an acid urine and display a chronic acidosis. Albright & Reifenstein have called attention to the fact

that they also excrete less ammonia than would be expected from the degree of systemic acidosis which is present. In a metabolic study of one of their patients these authors demonstrated that ammonia excretion did rise when urinary acidity was increased during an acidosis of even greater severity. If it is admitted that the urinary pH controls ammonia formation, then one can postulate that these patients are suffering from one defect in the metabolism of the distal tubular cell, rather than two, one involving acid formation and the other ammonia production.

SUMMARY

1. Urinary ammonia formation has been thought to be controlled by either (*a*) the acid-base composition of the plasma, or (*b*) the acidity of the urine.

2. By administration of an inhibitor of renal carbonic anhydrase these two factors have been made to vary discordantly, and further evidence was obtained about which one determines the rate of ammonia formation.

3. Urinary pH was elevated in rats by (*a*) giving *p*-sulphonamidobenzoic acid, and (*b*) the induction of a systemic alkalosis with $NaHCO_3$ or Na_2CO_3. Ammonia excretion remained a function of urinary pH in both groups of experiments.

4. The pH of the urine, i.e. the *intensity* of acidity, controls the amount of ammonia formed by the tubular cells per hour, but subsidiary factors must come into operation in certain circumstances.

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